Figure 1

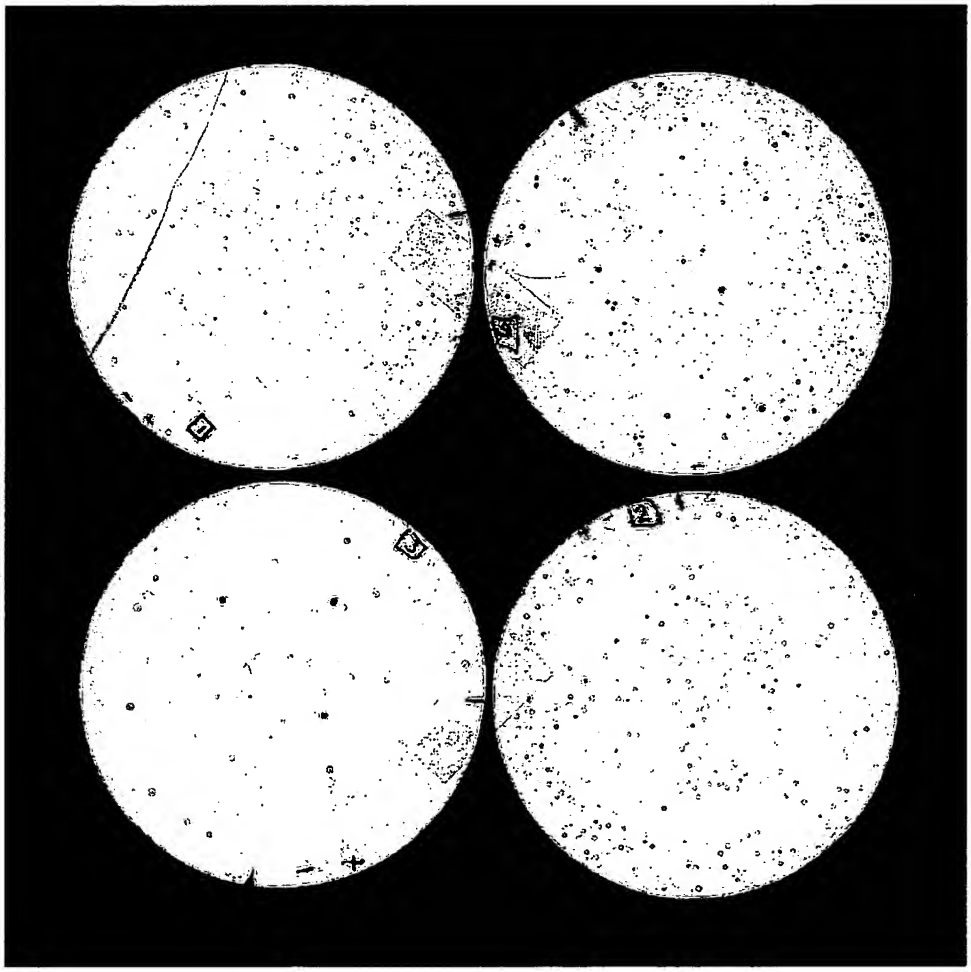

Figure 2

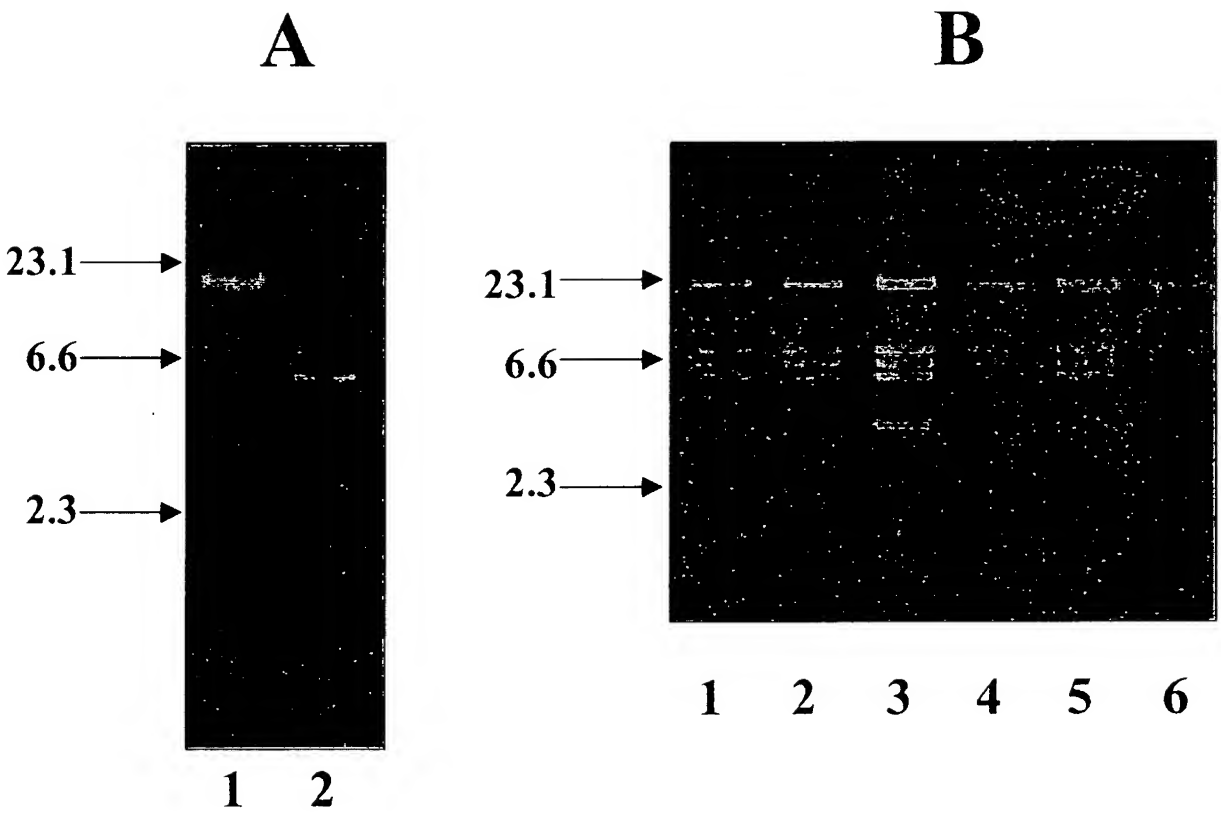

Figure 3

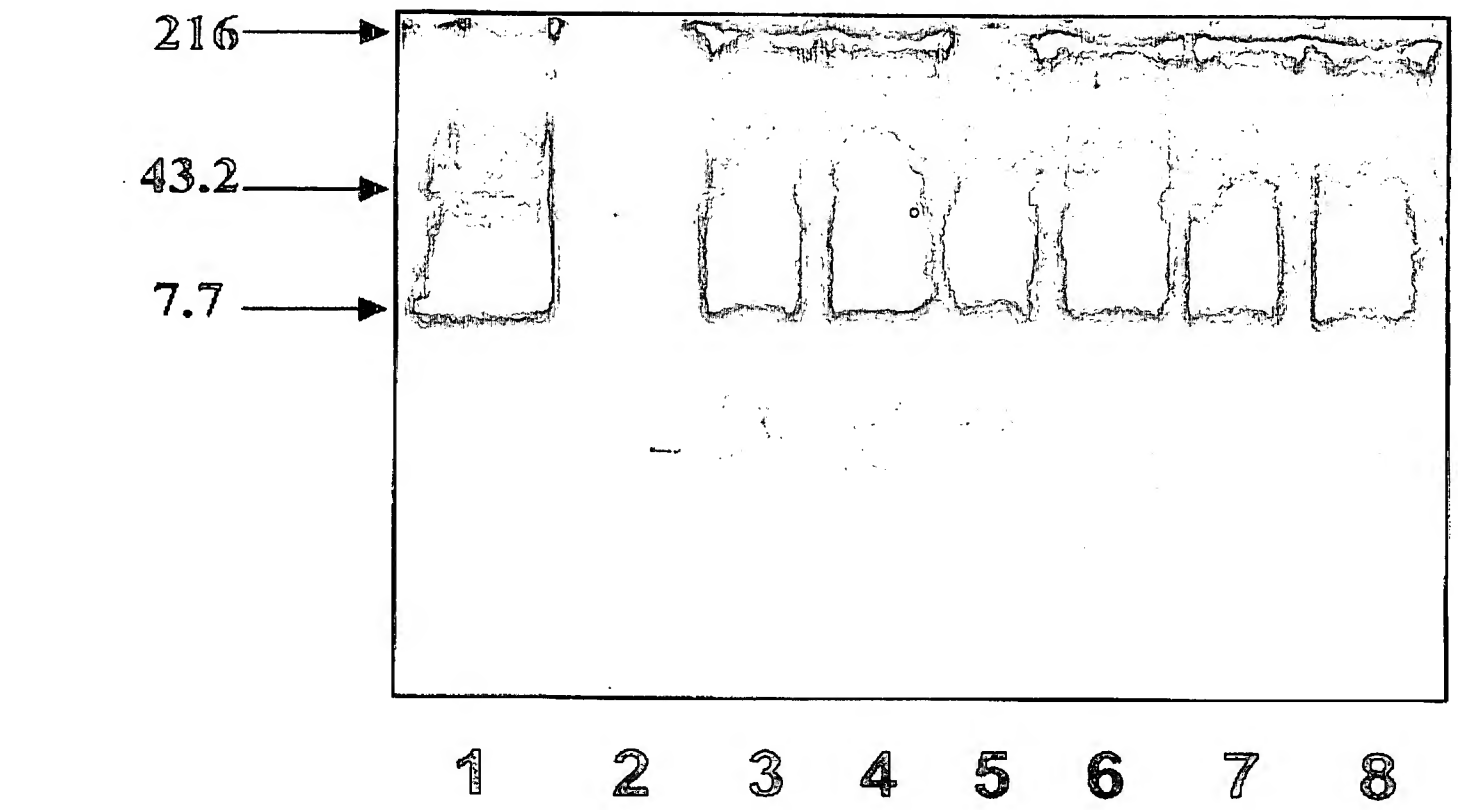

Figure 4

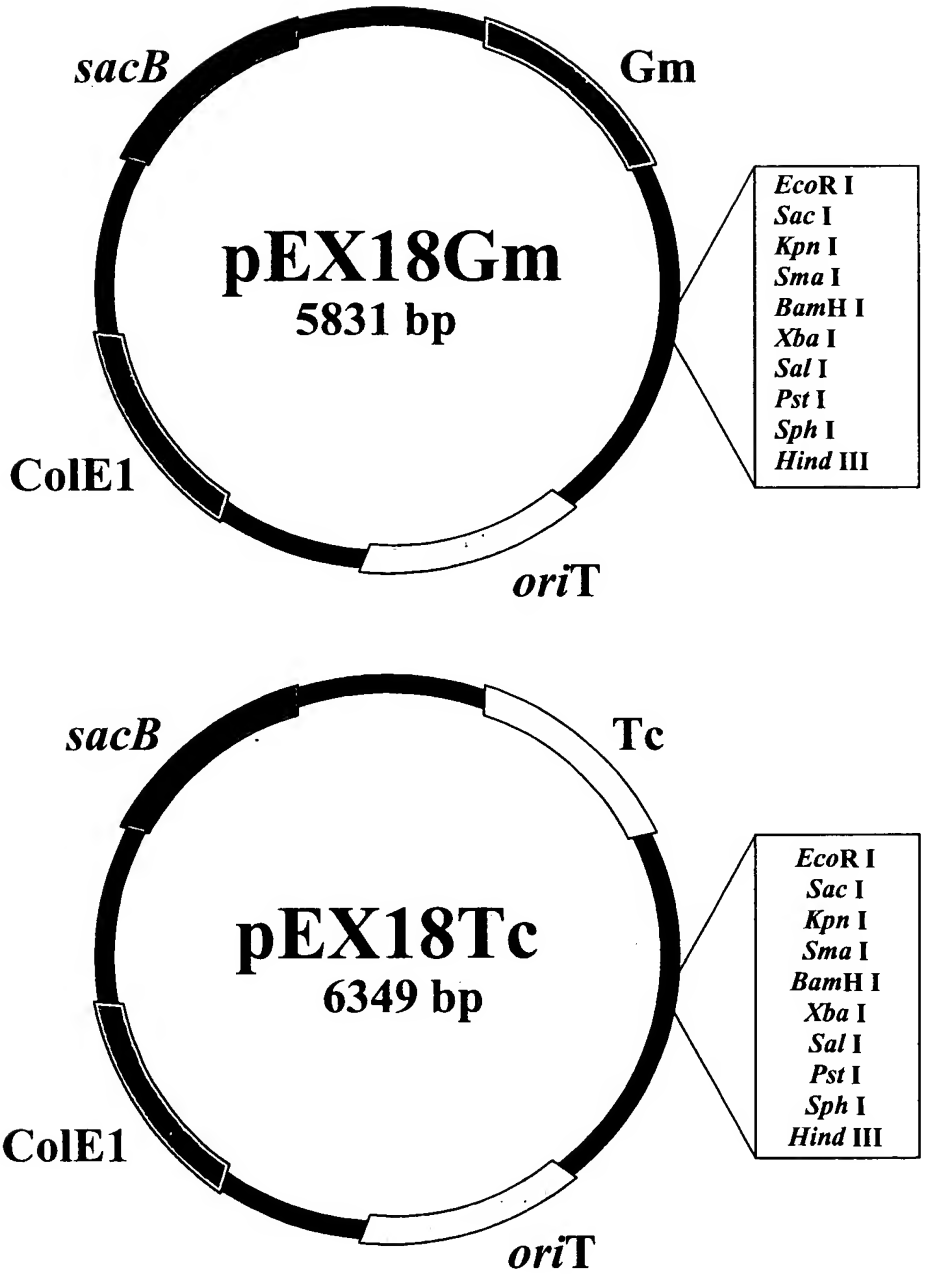
sacB
Gm
pEX18Gm
5831 bp
ColE1
oriT
EcoR I
Sac I
Kpn I
Sma I
BamH I
Xba I
Sal I
Pst I
Sph I
Hind III
sacB
Tc
pEX18Tc
6349 bp
ColE1
oriT
EcoR I
Sac I
Kpn I
Sma I
BamH I
Xba I
Sal I
Pst I
Sph I
Hind III

Figure 5

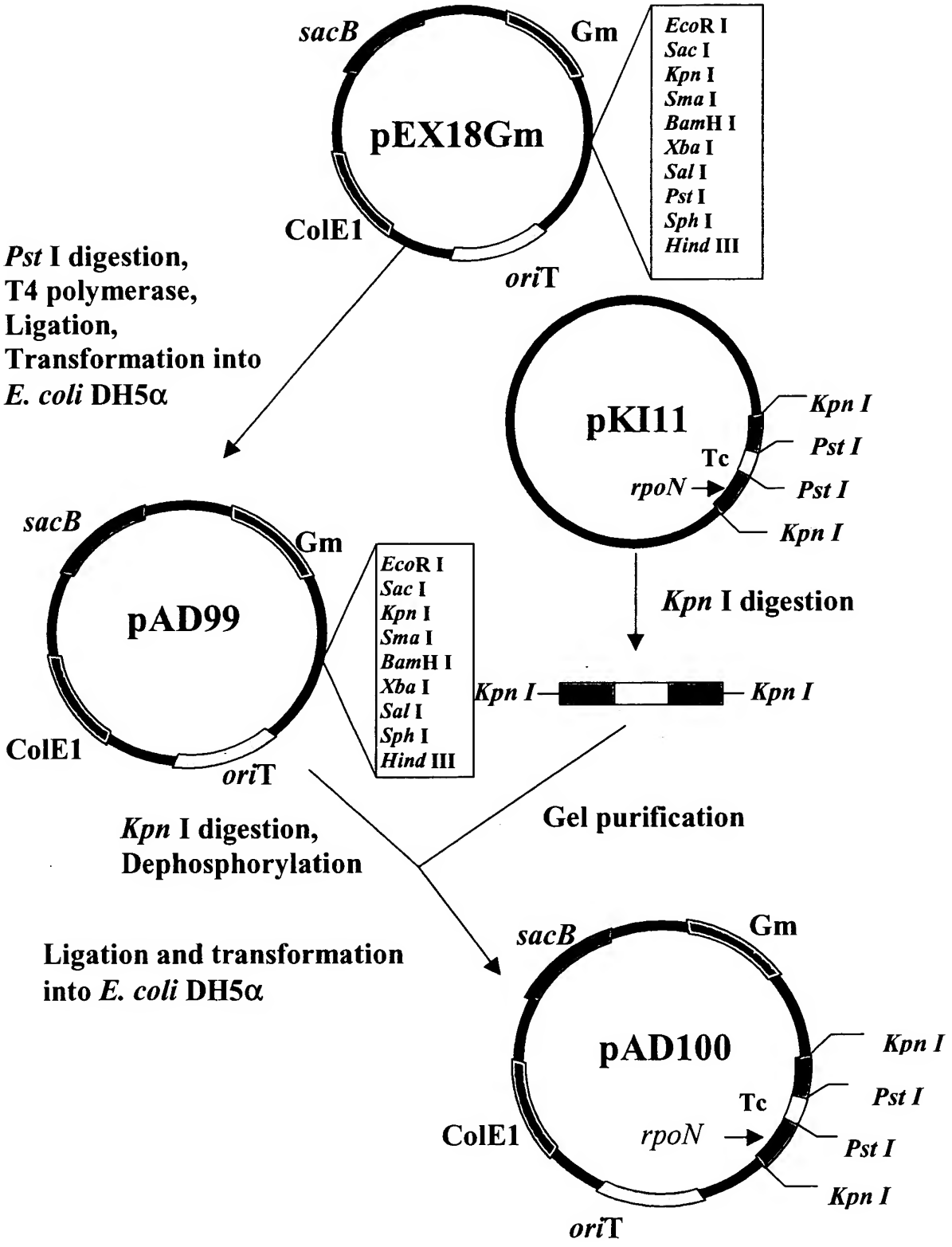
sacB
Gm
pEX18Gm
ColE1
oriT
EcoR I
Sac I
Kpn I
Sma I
BamH I
Xba I
Sal I
Pst I
Sph I
Hind III
Pst I digestion,
T4 polymerase,
Ligation,
Transformation into
E. coli DH5α
pKI11
Kpn I
Pst I
Tc
rpoN
Pst I
Kpn I
Kpn I digestion
sacB
Gm
pAD99
ColE1
oriT
EcoR I
Sac I
Kpn I
Sma I
BamH I
Xba I
Sal I
Sph I
Hind III
Kpn I
Kpn I
Kpn I digestion,
Dephosphorylation
Gel purification
Ligation and transformation
into E. coli DH5α
sacB
Gm
pAD100
ColE1
oriT
Kpn I
Pst I
Tc
rpoN
Pst I
Kpn I

Figure 6

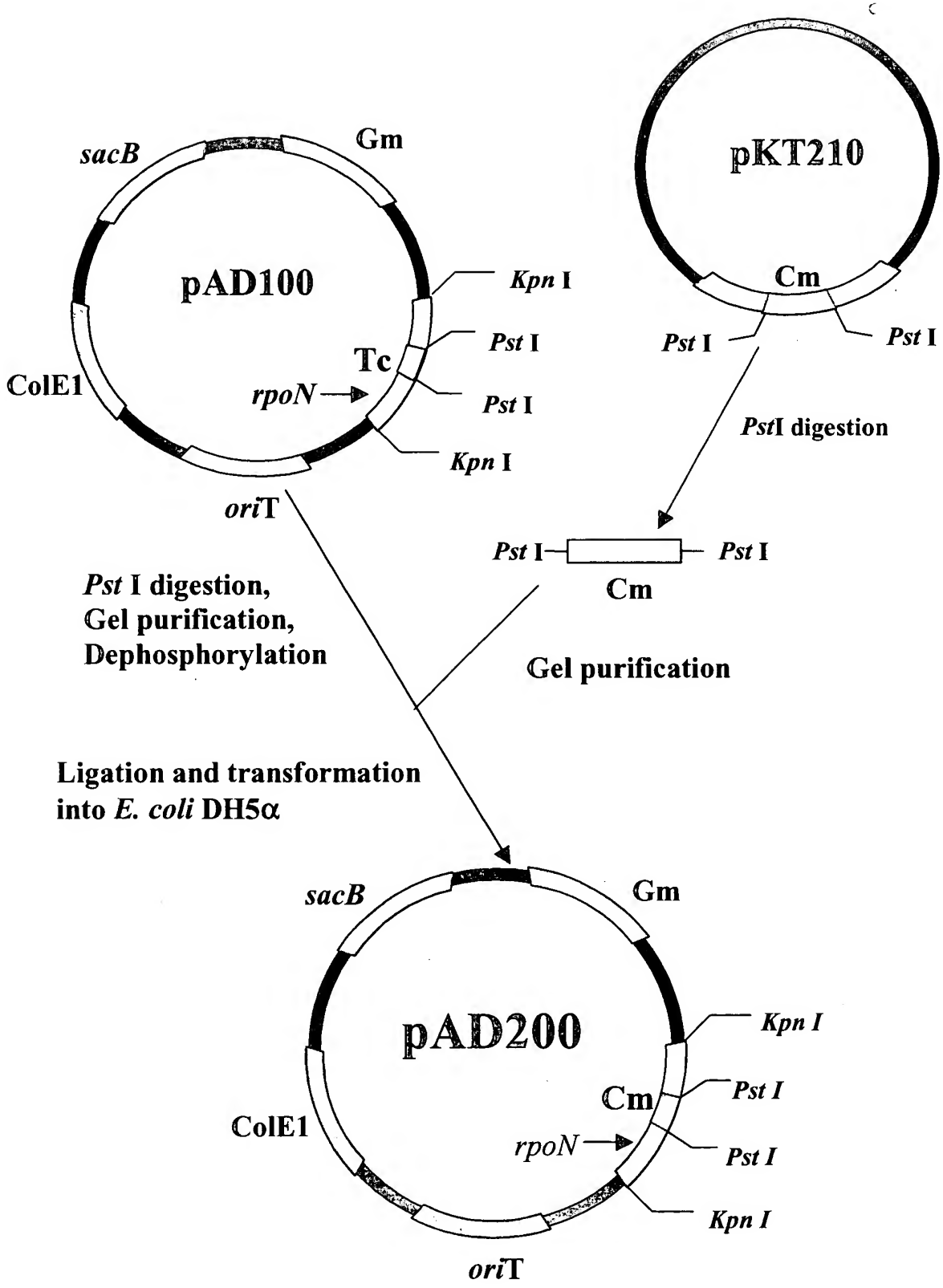
pKT210
Cm
Pst I
Pst I
PstI digestion
pAD100
sacB
Gm
Kpn I
Pst I
Tc
ColE1
rpoN
Pst I
Kpn I
oriT
Pst I digestion,
Gel purification,
Dephosphorylation
Pst I
Pst I
Cm
Gel purification
Ligation and transformation
into E. coli DH5α
pAD200
sacB
Gm
Kpn I
Cm
Pst I
ColE1
rpoN
Pst I
Kpn I
oriT

Figure 7

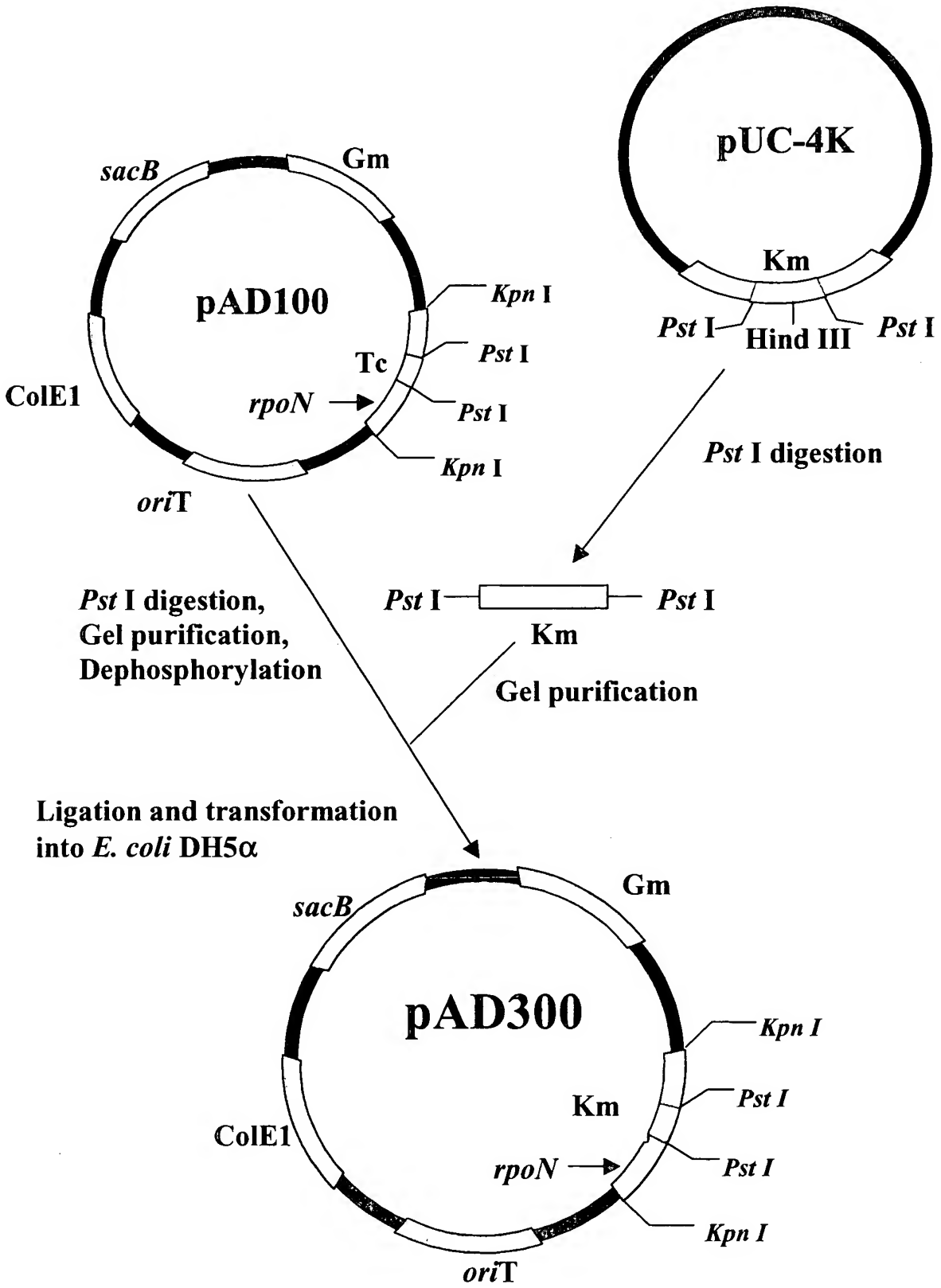
pUC-4K
Km
Pst I
Hind III
Pst I
sacB
Gm
pAD100
Kpn I
Pst I
Tc
ColE1
rpoN
Pst I
Kpn I
oriT
Pst I digestion
Pst I digestion,
Gel purification,
Dephosphorylation
Pst I
Pst I
Km
Gel purification
Ligation and transformation
into E. coli DH5α
sacB
Gm
pAD300
Kpn I
Pst I
Km
ColE1
rpoN
Pst I
Kpn I
oriT

Figure 8

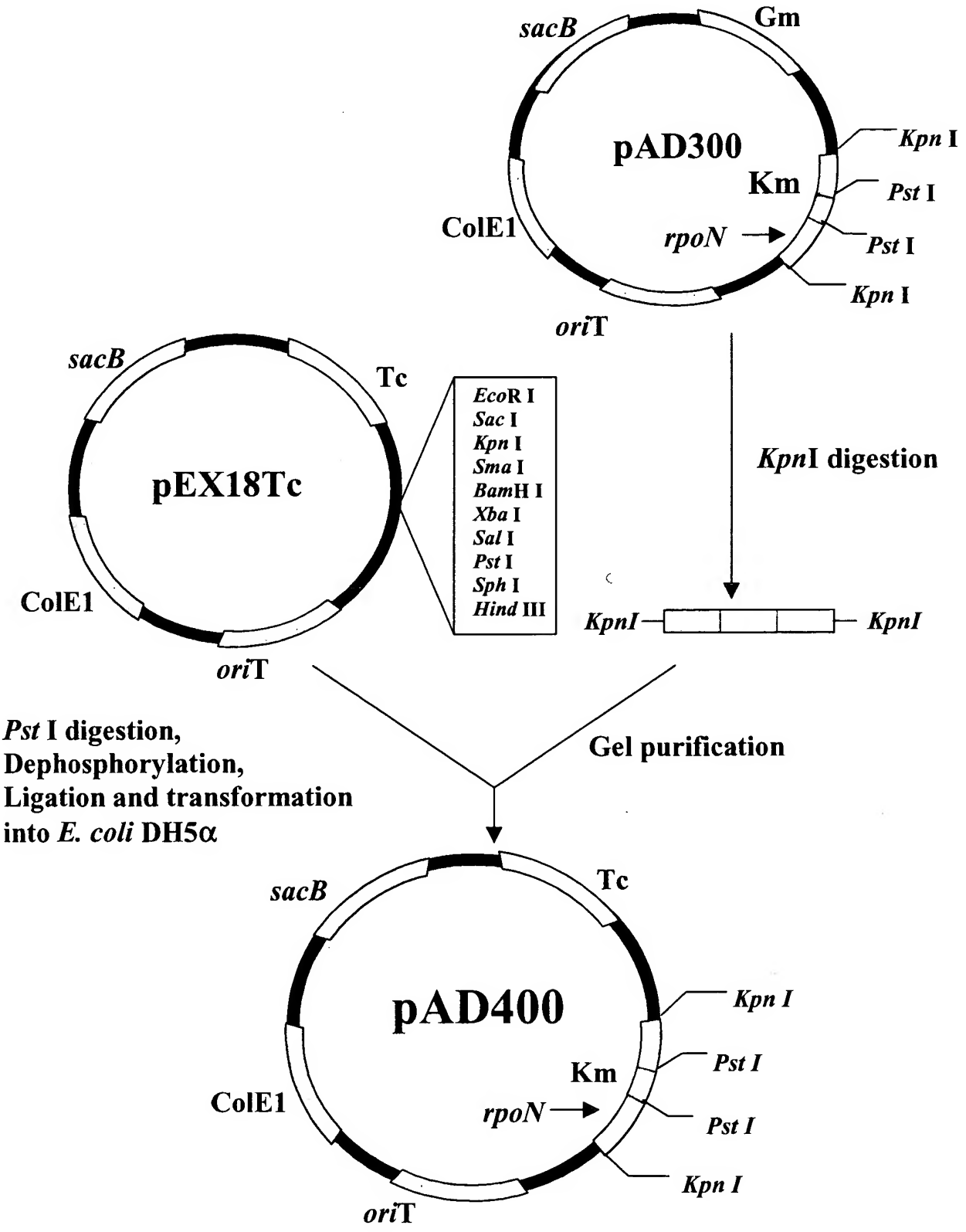
sacB
Gm
pAD300
Km
Kpn I
Pst I
Pst I
Kpn I
ColE1
rpoN
oriT
sacB
Tc
pEX18Tc
ColE1
oriT
EcoR I
Sac I
Kpn I
Sma I
BamH I
Xba I
Sal I
Pst I
Sph I
Hind III
KpnI digestion
KpnI
KpnI
Pst I digestion,
Dephosphorylation,
Ligation and transformation
into E. coli DH5α
Gel purification
sacB
Tc
pAD400
Km
Kpn I
Pst I
Pst I
Kpn I
ColE1
rpoN
oriT

Figure 9

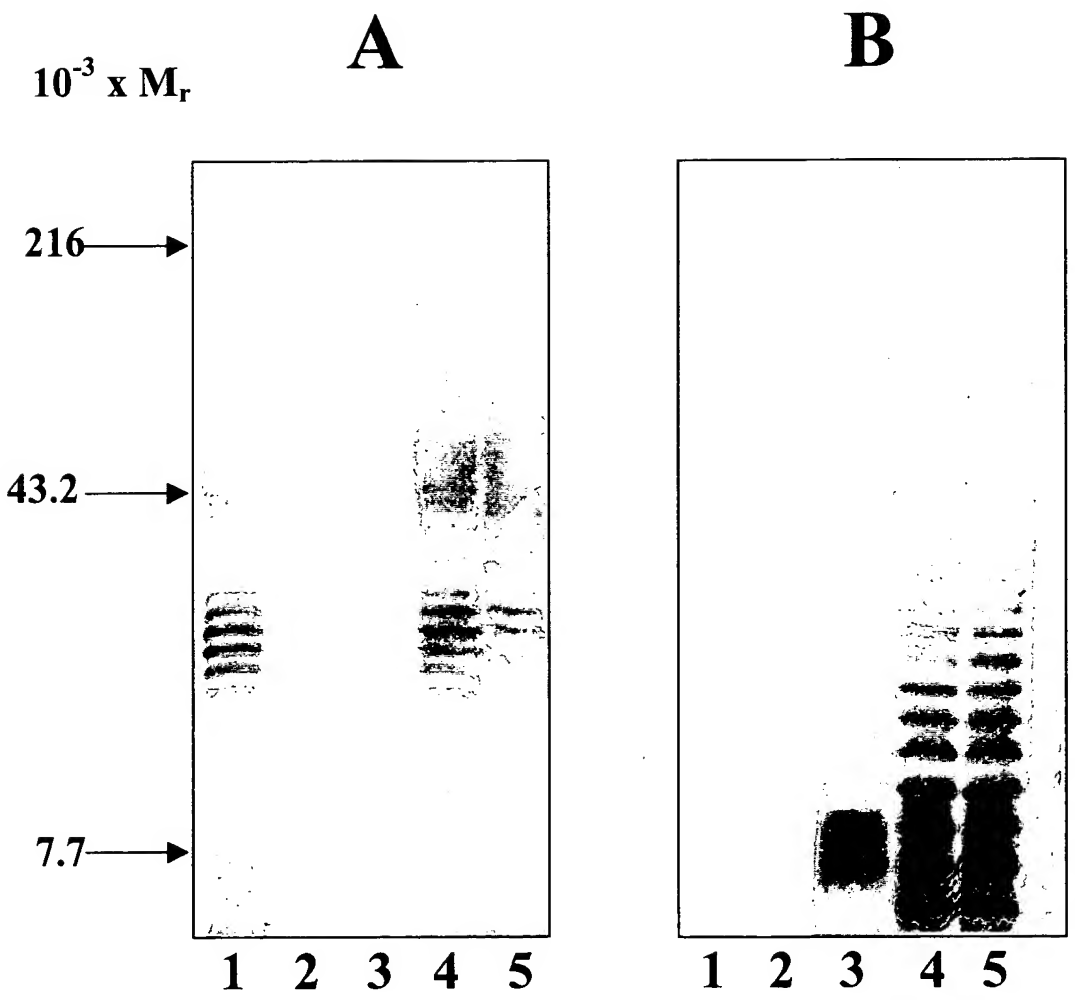

Figure 10

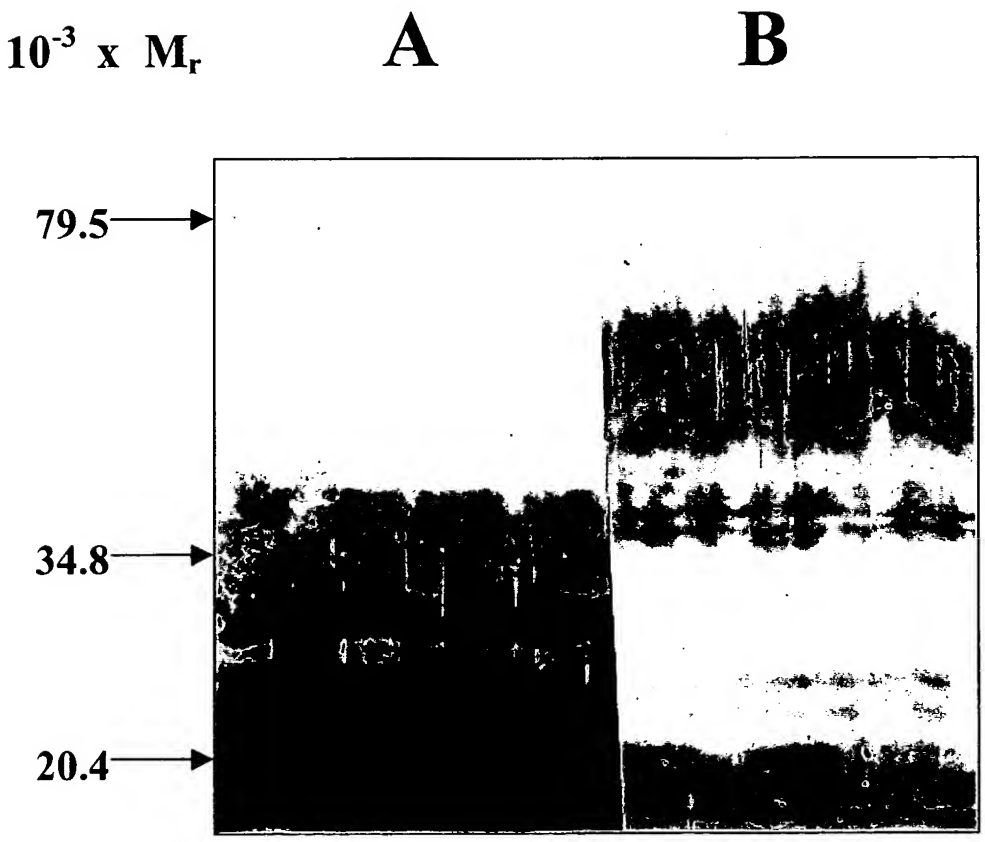

Figure 11

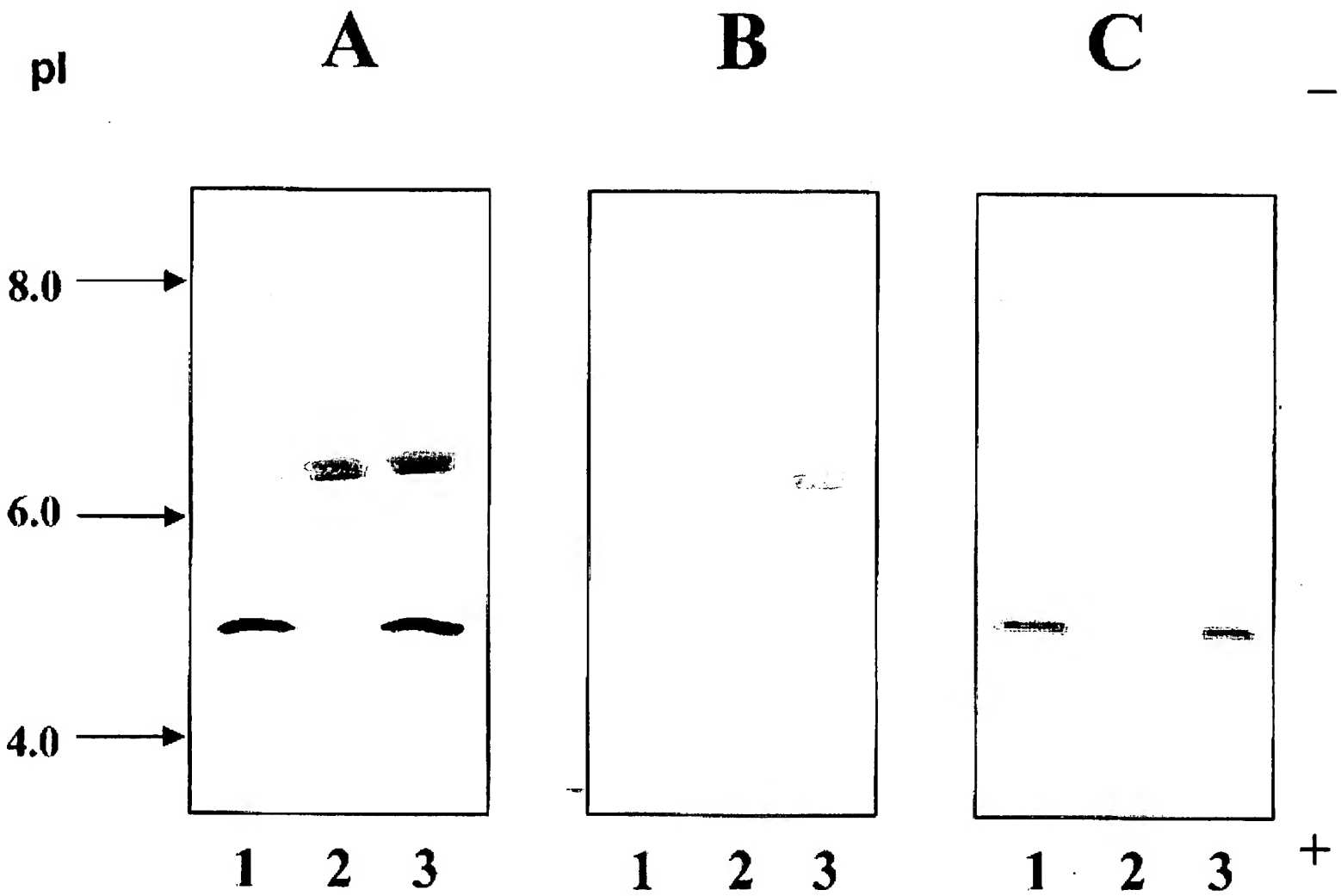

Figure 12

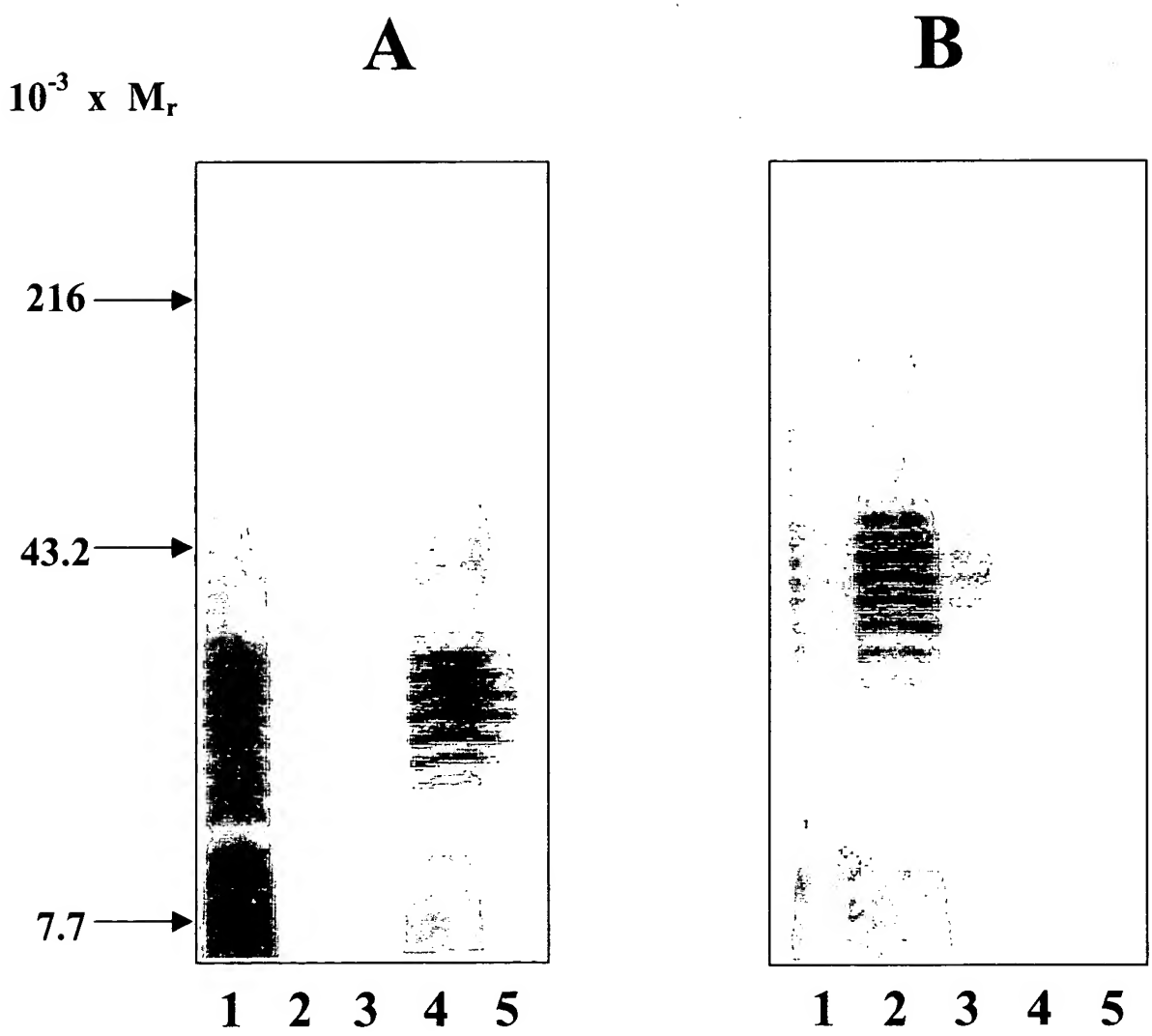

Figure 13

10^{-3} x M_r

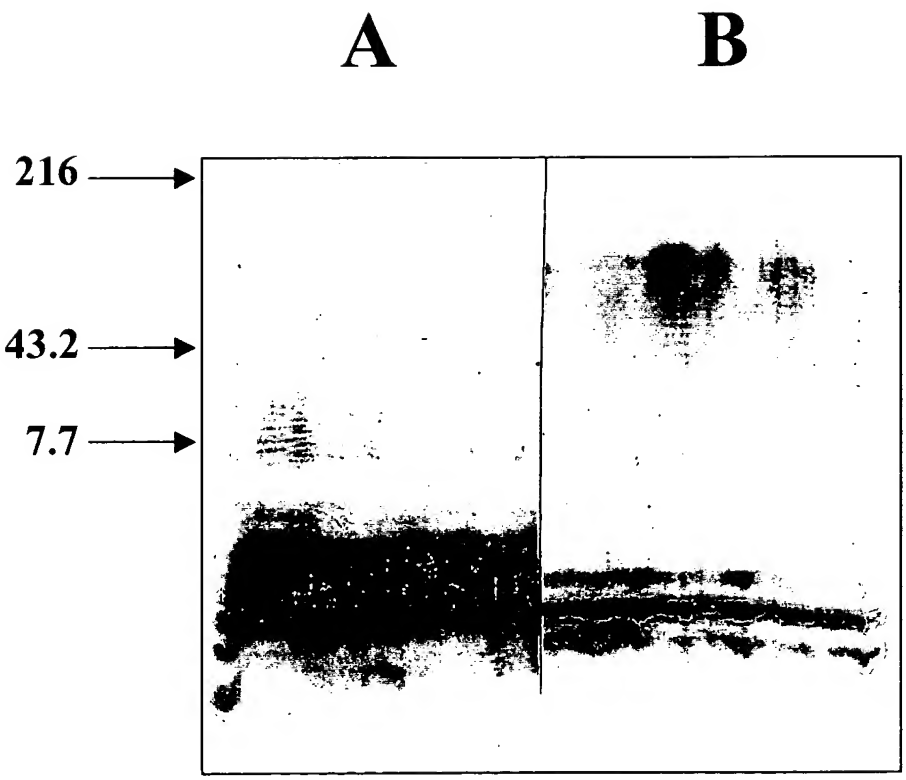

Figure 14

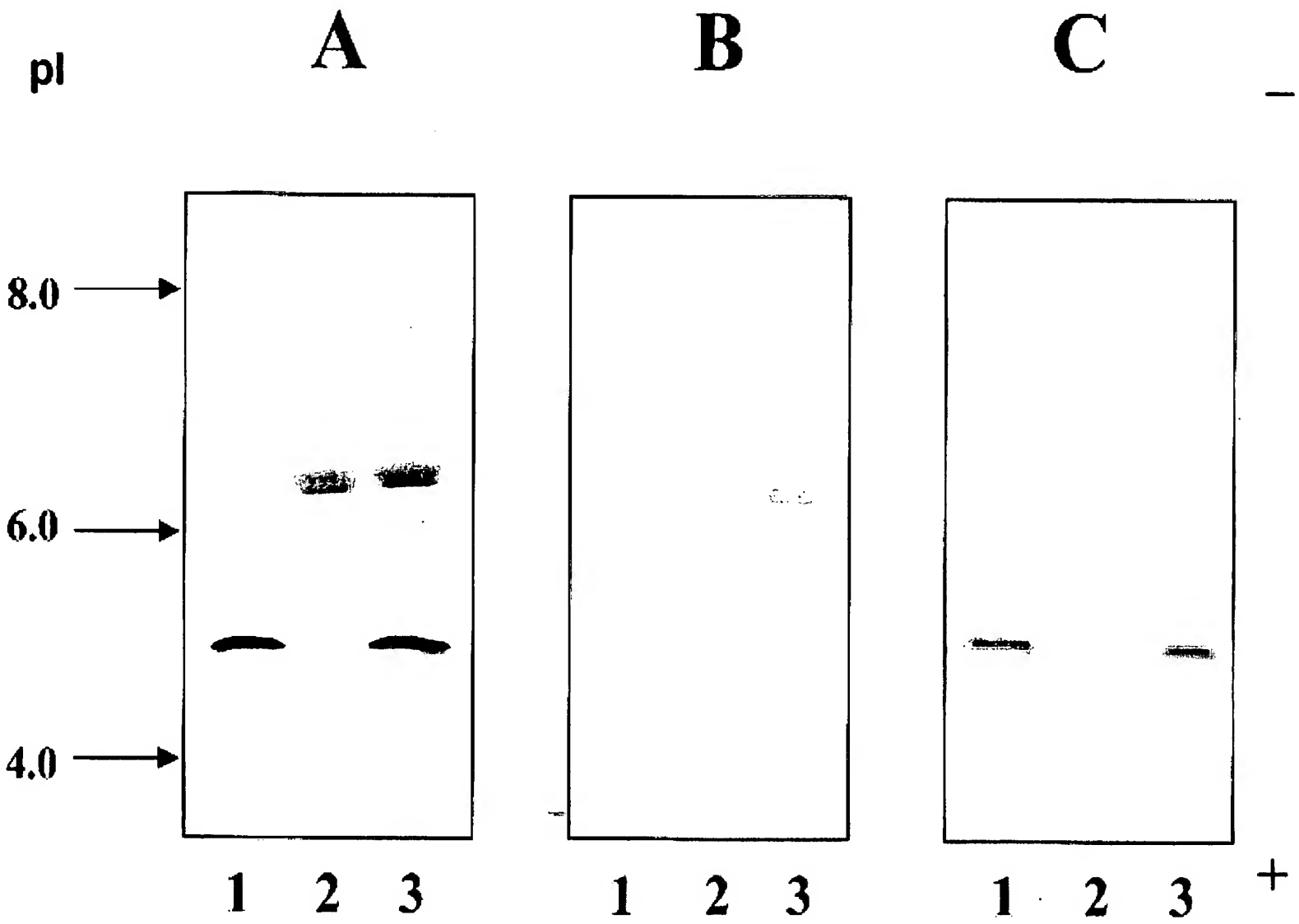

Figure 15

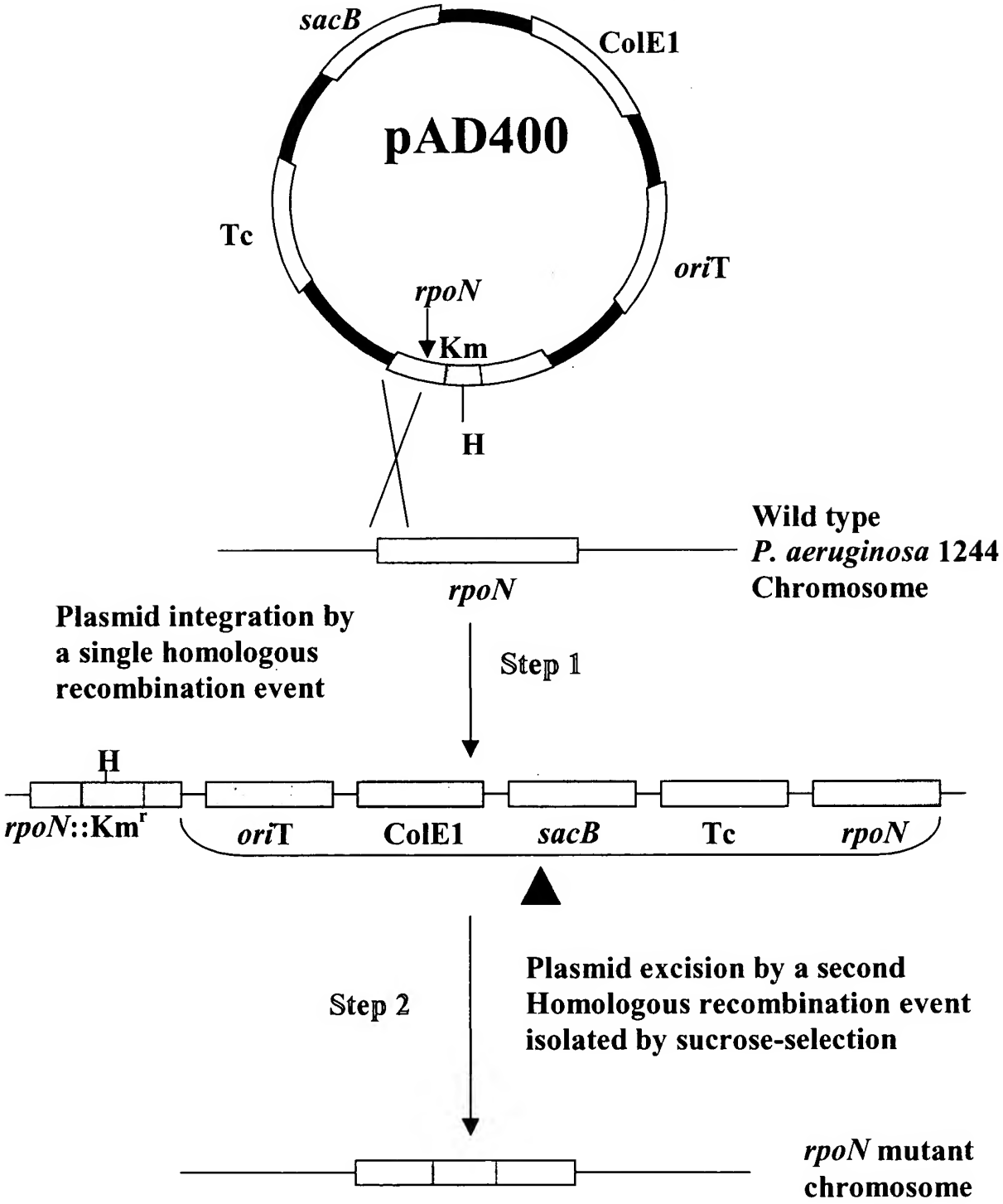
sacB
ColE1
pAD400
Tc
oriT
rpoN
Km
H
Wild type
P. aeruginosa 1244
Chromosome
rpoN
Plasmid integration by
a single homologous
recombination event
Step 1
H
rpoN::Km^r
oriT
ColE1
sacB
Tc
rpoN
Step 2
Plasmid excision by a second
Homologous recombination event
isolated by sucrose-selection
rpoN mutant
chromosome

Figure 16

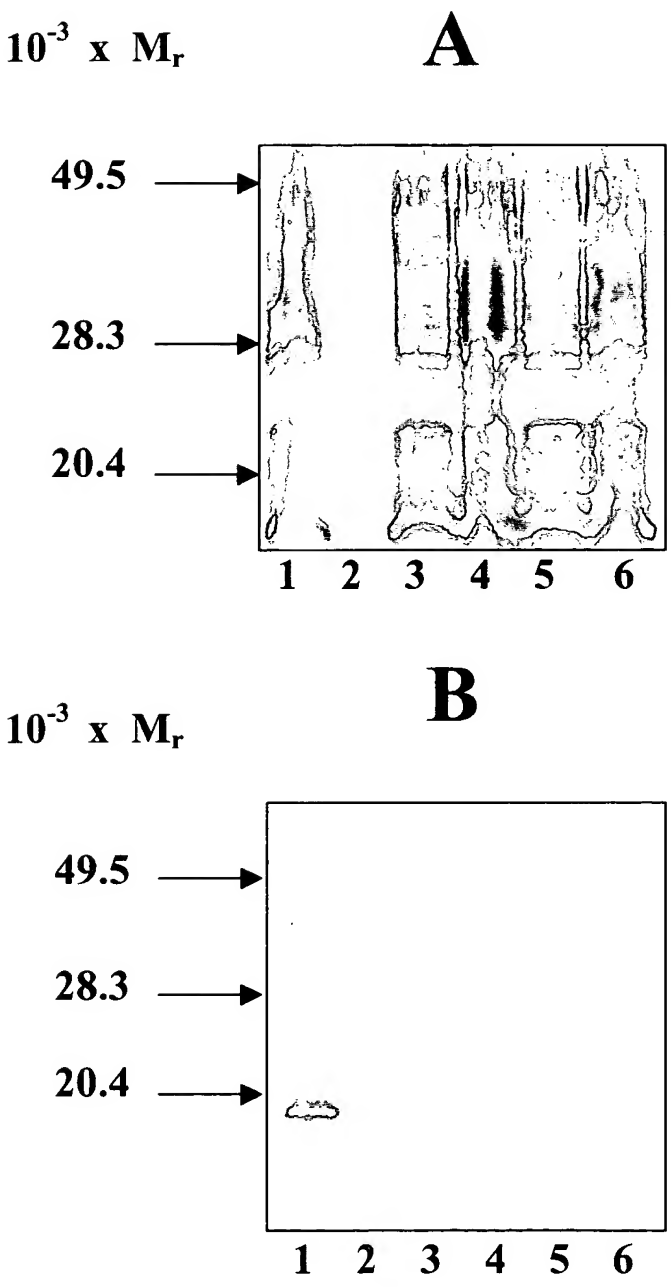

Figure 17a

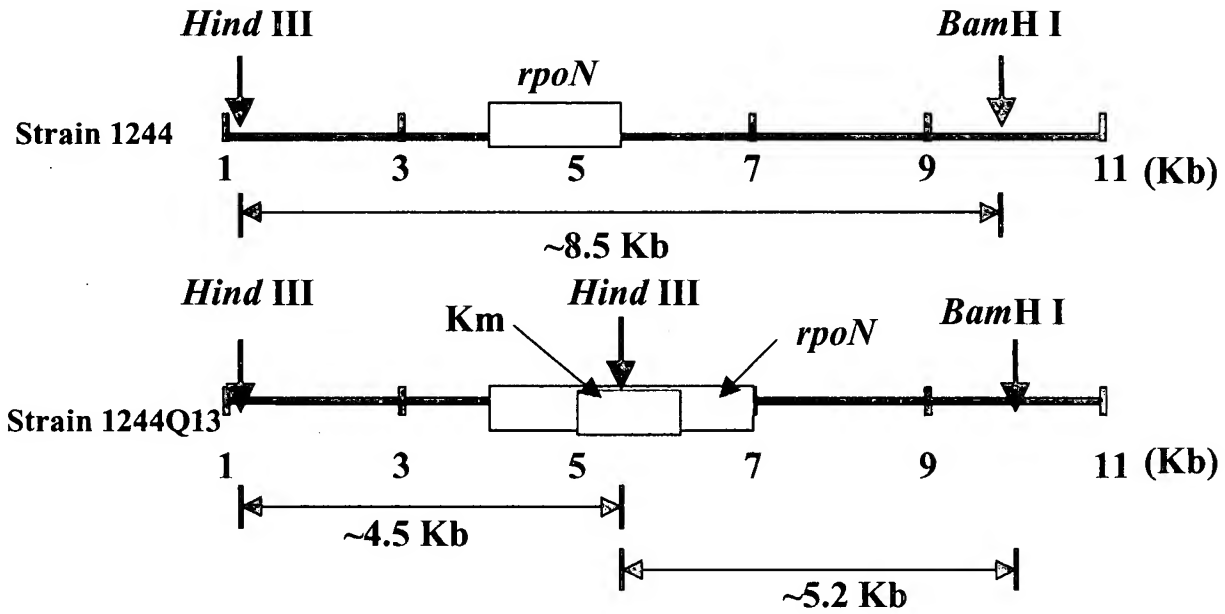
Hind III
BamH I
rpoN
Strain 1244
1
3
5
7
9
11 (Kb)
~8.5 Kb
Hind III
Km
Hind III
rpoN
BamH I
Strain 1244Q13
1
3
5
7
9
11 (Kb)
~4.5 Kb
~5.2 Kb

Figure 17b

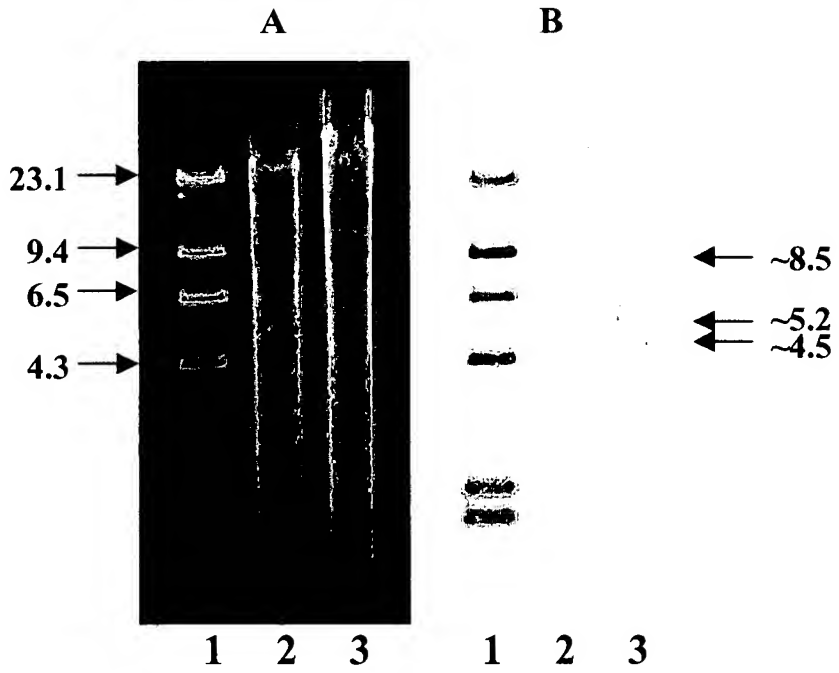

Figure 17c

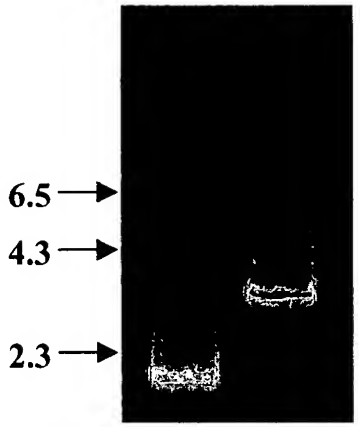

Figure 18

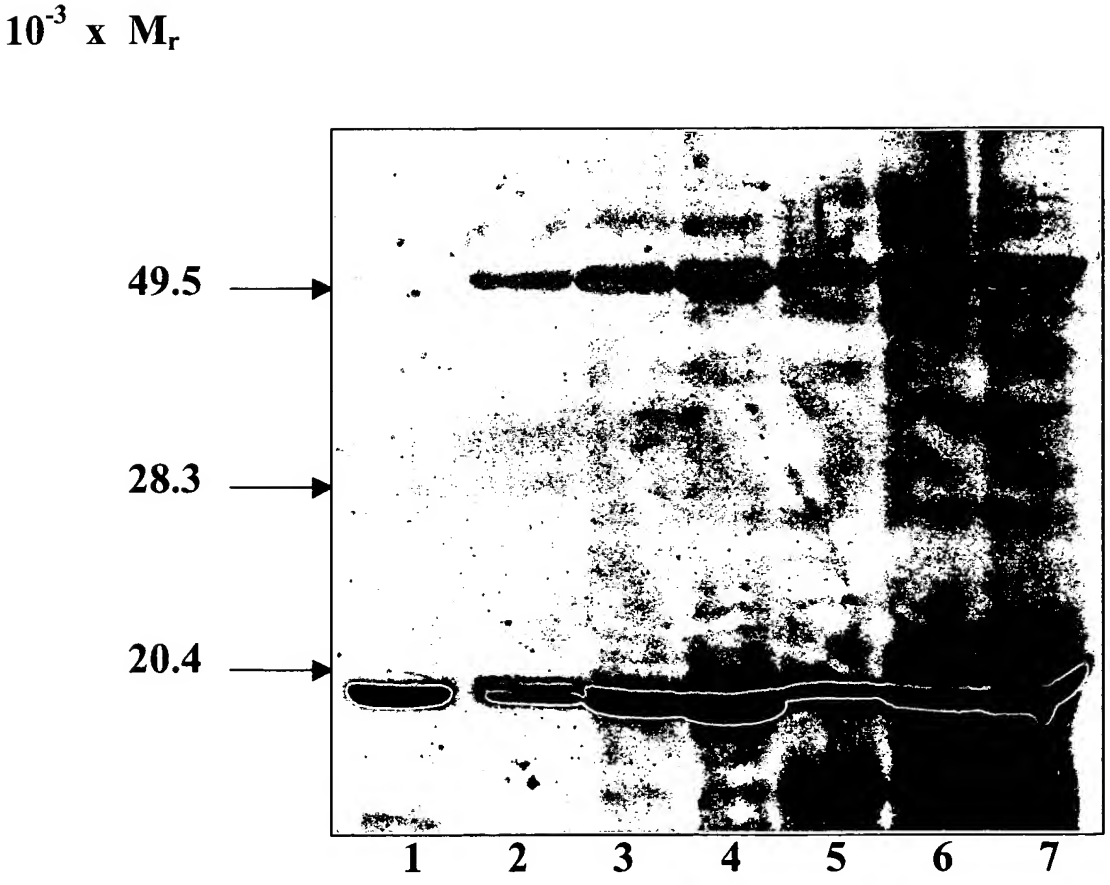
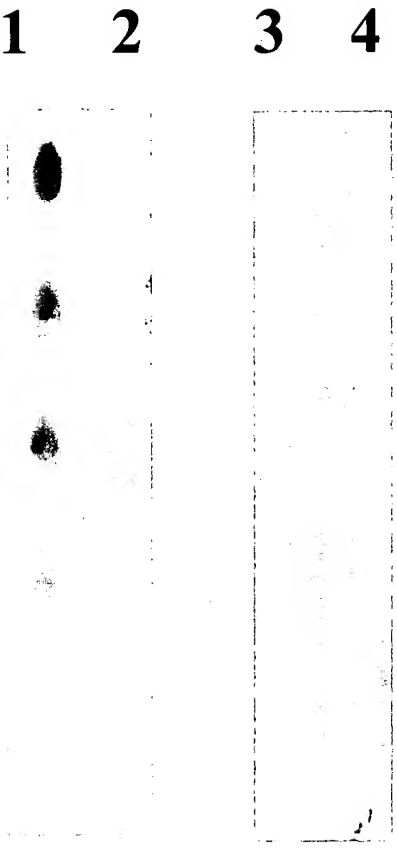
Figure 19